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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference VS:CE:FP17710	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International Application No. **PCT/AU2003/000415**	International Filing Date *(day/month/year)* 7 April 2003	Priority Date *(day/month/year)* 8 April 2002
International Patent Classification (IPC) or national classification and IPC **Int. Cl.** [7] A61K 38/04, A61K 39/395, A61K 38/08; A61P 13/12, A61P 9/10, A61P 11/00		
Applicant PROMICS PTY LIMITED et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

   [X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of **9** sheet(s).

3. This report contains indications relating to the following items:

   - I [X] Basis of the report
   - II [ ] Priority
   - III [ ] Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV [ ] Lack of unity of invention
   - V [X] Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI [ ] Certain documents cited
   - VII [ ] Certain defects in the international application
   - VIII [ ] Certain observations on the international application

Date of submission of the demand	Date of completion of the report
26 September 2003	2 July 2004
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer Arati Sardana **ARATI SARDANA** Telephone No. (02) 6283 2627

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/AU2003/000415

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed.

☒ the description, pages **2-4, 7-29, 31, 33-37 and 42** as originally filed,
pages , filed with the demand,
pages **1, 5, 6, 30 and 32** received on **01 June 2004** with the letter of **01 June 2004**

☒ the claims, pages , as originally filed,
pages , as amended (together with any statement) under Article 19,
pages , filed with the demand,
pages **38-41** received on **01 June 2004** with the letter of **01 June 2004**

☒ the drawings, pages **1/16-16/16** as originally filed,
pages , filed with the demand,
pages , received on with the letter of

☐ the sequence listing part of the description:
pages , as originally filed
pages , filed with the demand
pages , received on with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages

☐ the claims, Nos.

☐ the drawings, sheets/fig.

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

V. **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	**1-21**	**YES**
	Claims		**NO**
Inventive step (IS)	Claims	**2-21**	**YES**
	Claims	**1**	**NO**
Industrial applicability (IA)	Claims	**1-21**	**YES**
	Claims		**NO**

2. Citations and explanations (Rule 70.7)

CITATIONS:

D1: US 4,692,511 A

D2: AU 80926/98 A

D3: WO 02/14265 A

EXPLANATION:

NOVELTY:

Amended claims 1-21 are novel in light of the disclosure of documents D1 to D3.

INVENTIVE STEP (IS): Claim 1

The Attorney has argued in her submission with respect to US 4,692,511 that there are no experimental results to support the assertion that the compounds disclosed in the citation are effective for treatment of fibrotic condition.

However given the disclosure in US 4,692,511 that C5a receptor antagonist peptides disclosed there in are particularly useful in the treatment of fibrotic condition idiopathic pulmonary fibrosis, the skilled person would reasonably be expected to use peptides of US 4,692,511 in the treatment of fibrosis with a reasonable expectation of success. Therefore claim 1 would still lack an inventive step.

THERAPEUTIC METHOD

FIELD OF THE INVENTION

This application claims priority from Australian provisional patent application No. PS1606, filed on 8 April 2002.

This invention relates to the use of an antagonist of a G protein-coupled receptor in the prevention and/or treatment of fibrosis, such as the treatment of fibrosis associated with myocardial infarction, diabetes, or certain pulmonary conditions. In a preferred embodiment the antagonist is a C5a receptor antagonist, more preferably a cyclic peptide antagonist of the C5a receptor.

BACKGROUND OF THE INVENTION

All references, including any patents or patent applications, cited in this specification are hereby incorporated by reference. No admission is made that any reference constitutes prior art. The discussion of the references states what their authors assert, and the applicants reserve the right to challenge the accuracy and pertinency of the cited documents. It will be clearly understood that, although a number of prior art publications are referred to herein, this reference does not constitute an admission that any of these documents forms part of the common general knowledge in the art, in Australia or in any other country.

G protein-coupled receptors are prevalent throughout the human body, comprising approximately 60% of known cellular receptor types. They mediate signal transduction across the cell membrane for a very wide range of endogenous ligands and consequently participate in a diverse array of physiological and pathophysiological processes, including, but not limited to, those associated with cardiovascular, central and peripheral nervous system reproductive, metabolic, digestive, immunoinflammatory, and growth disorders, as well as other cell regulatory and proliferative disorders. Agents which selectively modulate

et al. 1997).

The effects of drug-induced and hypertension-induced pulmonary and renal fibrosis in animal models can be prevented or partially reversed by compounds which act by suppressing inflammatory events and down-regulating lung pro-collagen I over-expression (Iyer et al., 1999a,b).

We have shown that the administration of pirfenidone or spironolactone can prevent and partially reverse cardiac fibrosis and the increase in cardiac stiffness which occurs in streptozotocin-induced diabetes in rats (Miric G, et al., 2001) It is thought that pirfenidone acts by inhibiting increased TGF-β mRNA expression, allowing an increase in expression of metalloproteases which degrade the collagen I laid down during fibrosis. The mode of action of spironolactone is at present unknown. Spironolactone is a steroid analogue which is primarily used as a diuretic; pirfenidone (5-methyl-1-phenyl-2-(1H)-pyridone), an investigational compound being investigated as an anti-fibrotic agent in a number of indications.

It would be highly desirable to identify other therapeutically or prophylactically active agents for use in the treatment or prevention of fibrosis.

SUMMARY OF THE INVENTION

The overexpression or underregulation of a G-protein-coupled receptor, the C5a receptor, has been implicated in immune-system mediated events such as inflammation. Agents which influence C5a receptor activity, such as C5a receptor antagonists, have the potential to mediate inflammatory events, and may provide a means of therapeutic or prophylactic intervention, but have not previously been suggested as potential agents in the treatment or prevention of fibrosis.

We have now surprisingly found that a cyclic peptide with C5a receptor antagonist has the ability to

ameliorate cardiac fibrosis in an animal model of this condition.

According to a first aspect, the invention provides a method of prevention, treatment or alleviation of a fibrotic condition, comprising the step of administering an effective amount of an antagonist of a G protein-coupled receptor to a subject in need of such treatment.

The use of any compound having activity as an antagonist of a G protein-coupled receptor, and particularly as a C5a receptor antagonist, is contemplated, including but not limited to those disclosed in our earlier International patent applications No.PCT/AU98/00490 or No. PCT/AU02/01427 or in International patent applications No. PCT/US00/11187 by Neurogen Corporation and No. PCT/JP01/06902 by Welfide Corporation, or antibody antagonists such as those disclosed in PCT/US00/24219 or US patent No. 6355245. The entire disclosures of all of these specifications are incorporated herein by this cross-reference.

More preferably the C5a receptor antagonist is a peptide or a peptidometic compound, and more preferably is a cyclic peptide or a cyclic peptidometic compound. Even more preferably the compound is a cyclic peptide or a cyclic peptidometic compound of PCT/AU98/00490 or PCT/AU02/01427.

Still more preferably the antagonist is a compound which

(a) is an antagonist of a G protein-coupled receptor,
(b) has substantially no agonist activity, and
(c) is a cyclic peptide or peptidomimetic compound of formula I

Xylocaine to prevent airway spasm, the rats were intubated and a slow injection of bleomycin or saline control was completed. The rats were then rotated gently for about 1-2 minutes to allow the solution to diffuse evenly into both lungs (Christensen et al 2000). Rats were kept in the fume cupboard until totally recovered, and then monitored for up to 18 days. Body weight, food and water intake, and respiration were monitored daily.

Respiration was elevated as follows: Score 0, normal respiration; Score 1, increased rate of breathing; and Score 2, mouth open respiration. Rats were euthanased before the end of the experimental period, if they consistently lost more than 10% body weight for 48 hours, had Score 2 respiration or had Score 1 respiration for 48 hours.

At the end of this period the rats were killed by exsanguination under anaesthesia, so that the lungs were clear of blood. For each rat, the left lung was immediately frozen in liquid nitrogen and stored at -20^{0}C for quantitative collagen analysis using hydroxyproline assay. The right lung was fully inflated and fixed with 10% formulated formalin by airway gravity fixation at a pressure of 30 cm water for 1 minute. Haematoxylin and eosin (H&E) and Picro Sirius Red (PR) staining for collagen were performed to assess collagen deposition in the lung. For quantitation of collagen stained with PR, polarized light images were converted to grey scale, and the total number of white pixels (specific for collagen) per image was determined as a percentage of the total pixel area. The procedure was applied to a total of four fields in the alveolar area and two fields in the peribronchial area and blood vessels per sample (Wang et al, 2000). The largest lobe of the right lung (from 4 lobes) in each rat was chosen. The data was analysed using the program "Sion Image".

Hydroxyproline assay was performed by the method

Table 1.
Lung weight and body weight in bleomycin-induced pulmonary fibrosis (7-9 days)

Condition	Left lung weight (g)	Body weight (g)	Ratio $x10^{-3}$
Normal	0.507 ± 0.003	240.6 ± 4.667	1.9 ± 0.36
Bleomycin	1.004 ± 0.04	226 ± 8.083	4.47 ± 0.46**
Bleomycin + PMX53	0.974 ± 0.132	228 ± 7.583	4.25 ± 1.07**

**: P<0.001, n=3, compared to normal rats.

Under the microscope, numbers of inflammatory cells, including PMNs, macrophages, lymphocytes etc. were observed in the alveolar spaces, with massive leakage of plasma and red blood cells; this is illustrated in Figure 13a. The size and number of type II AECs in the alveolar spaces was clearly increased, as shown in Figure 13b, while in normal lung, the type II AECs covered only 5 - 10% of the surface area of the alveoli, as shown in Figure 14.

There was no significant difference in histology between drug-treated and non-treated groups. Collagen deposition in bleomycin instillation lungs showed a significant increase compared to normal lungs (P<0.01, n=3); saline instillation lungs (P<0.01, n=3); and saline instillation with PMX53-treated lungs (P<0.01, n=3). However, there was no significant difference between the drug-treated group and non-treated group (P>0.01, n=4). These results are summarised in Figure 15.

2. Pulmonary fibrosis

Eighteen days after intra-tracheal instillation of bleomycin, the degree of oedema was reduced in bleomycin-instilled lungs, and the lung/body weight ratio did not

CLAIMS

1. A method of prevention, treatment or alleviation of a fibrotic condition, comprising the step of administering an effective amount of an antagonist of a C5a receptor to a subject in need of such treatment, in which the antagonist is a peptide or a peptidomimetic compound.

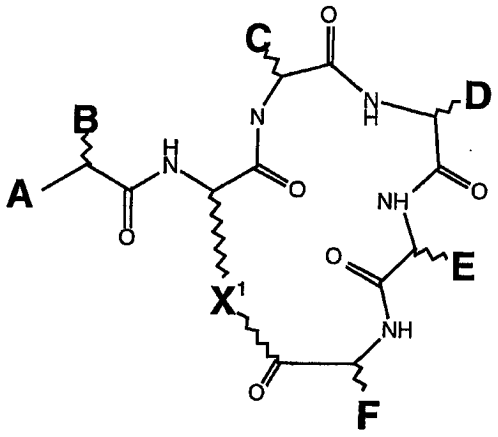
2. A method according to claim 1, in which the antagonist is a cyclic peptide or a cyclic peptidomimetic compound.

3. A method according to claim 1 or claim 2, in which the inhibitor is a compound which

a) is an antagonist of a G protein-coupled receptor,

b) has substantially no agonist activity, and

c) is a cyclic peptide or peptidomimetic compound of formula I



where A is H, alkyl, aryl, NH_2, NH-alkyl, $N(alkyl)_2$, NH-aryl, NH-acyl, NH-benzoyl, $NHSO_3$, $NHSO_2$-alkyl, $NHSO_2$-aryl, OH, O-alkyl, or O-aryl;

B is an alkyl, aryl, phenyl, benzyl, naphthyl or indole group, or the side chain of a D- or L-amino acid, but is not the side chain of glycine, D-phenylalanine, L-

homophenylalanine, L-tryptophan, L-homotryptophan, L-tyrosine, or L-homotyrosine;

C is the side chain of a D-, L- or homo-amino acid such as glycine, alanine, leucine, valine, proline, hydroxyproline, or thioproline, but is not the side chain of isoleucine, phenylalanine, or cyclohexylalanine;

D is the side chain of a neutral D-amino acid, but is the side chain of glycine or D-alanine, a bulky planar side chain, or a bulky charged side chain;

E is a bulky substituent, but is not the side chain of D-tryptophan, L-N-methyltryptophan, L-homophenylalanine, L-2-naphthyl L-tetrahydroisoquinoline, L-cyclohexylalanine, D-leucine, L-fluorenylalanine, or L-histidine;

F is the side chain of L-arginine, L-homoarginine, L-citrulline, or L-canavanine, or a bioisostere thereof; and

X is $-(CH_2)_nNH-$ or $(CH_2)_n-S-$, where n is an integer of from 1 to 4; $-(CH_2)_2O-$; $-(CH_2)_3O-$; $-(CH_2)_3-$; $-(CH_2)_4-$; $-CH_2COCHRNH-$; or $-CH_2-CHCOCHRNH-$, where R is the side chain of any common or uncommon amino acid.

4. A method according to claim 3, in which n is 2 or 3.

5. A method according to claim 3 or claim 4, in which A is an acetamide group, an aminomethyl group, or a substituted or unsubstituted sulphonamide group.

6. A method according to claim 5, in which A is a substituted sulphonamide, and the substituent is an alkyl chain of 1 to 6, or a phenyl or toluyl group.

7. A method according to claim 6, in which the substituent is an alkyl chain of 1 to 4 carbon atoms.

8. A method according to any one of claims 3 to 7, in which B is the side chain of L-phenylalanine or L-phenylglycine.

9. A method according to any one of claims 3 to 8, in which C is the side chain of glycine, alanine, leucine,

valine, proline, hydroxyproline, or thioproline.

10. A method according to any one of claims 3 to 9, in which D is the side chain of D-Leucine, D-homoleucine, D-cyclohexylalanine, D-homocyclohexylalanine, D-valine, D-norleucine, D-homo-norleucine, D-phenylalanine, D-tetrahydroisoquinoline, D-glutamine, D-glutamate, or D-tyrosine.

11. A method according to any one of claims 3 to 10, in which the antagonist is a compound which has antagonist activity against C5aR, and has no C5a agonist activity.

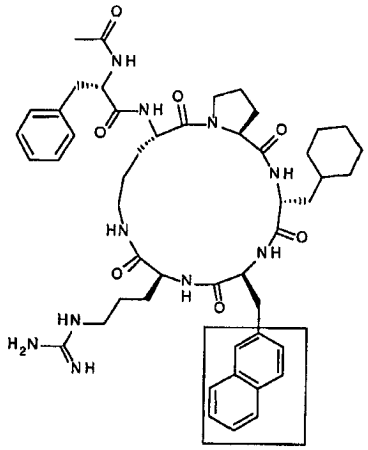
12. A method according to any one of claims 1 to 11, in which the inhibitor has potent antagonist activity at sub-micromolar concentrations.

13. A method according to any one of claims 1 to 12, in which the compound has a receptor affinity IC50<25μM, and an antagonist potency IC50<1μM.

14. A method according to any one of claims 1 to 13, in which the compound is selected from the group consisting of compounds **1** to **6**, **10** to **15**, **17**, **19**, **20**, **22**, **25**, **26**, **28**, **30**, **31**, **33** to **37**, **39** to **45**, **47** to **50**, **52** to **58** and **60** to **70** described in International patent application No.PCT/AU02/01427.

15. A method according to claim 14, in which the compound is AcF[OP-DCha-WR] (PMX53 compound **1**), AcF[OP-DPhe-WR] (compound **33**), AcF[OP-DCha-FR] (compound **60**) or AcF[OP-Dcha-WCit] (compound **45**).

16. A method according to claim 15, in which the compound is PMX53, having the formula



17. A method according to any one of claims 1 to 16, in which the fibrotic condition is selected from the group consisting of multiple sclerosis, proliferative vitroretinopathy, macular degeneration, scleroderma, sclerosing peritonitis, fibrosis arising from trauma, burns, chemotherapy, radiation, infection or surgery and fibrosis of the kidney, liver, heart or lungs, chronic hypertension and diabetes mellitus.

18. A method according to claim 17, in which the fibrotic condition is cardiac fibrosis or pulmonary fibrosis.

19. The use of a C5a receptor antagonist as defined in any one of claims 1 to 16 for the manufacture of a medicament for use in the treatment of a fibrotic condition.

20. A use according to claim 19, in which the fibrotic disorder is selected from the group consisting of multiple sclerosis, proliferative vitroretinopathy, macular degeneration, scleroderma, sclerosing peritonitis, fibrosis arising from trauma, burns, chemotherapy, radiation, infection or surgery and fibrosis of the kidney, liver, heart or lungs, chronic hypertension and diabetes mellitus.

21. A use according to claim 20, in which the fibrotic condition is cardiac fibrosis or pulmonary fibrosis.